Natural Suppression of Soilborne Plant Diseases

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摘　　要

柯文雄（1985）抑制土壤傳染病害的自然方法　（美國夏威夷大學）

根據土壤樣品分析，*Pythium splendens* 的孢囊發芽率與土壤的 pH 值成負相關。高含量的鈣和高密度的微生物是抑病的原因。在已測定過的土壤樣品中，*Rhizoctonia solani* 的菌絲生長與土壤 pH 值成正相關。抑菌作用乃是由微生物的活動及高量的鋁離子所引起。一個由森林地所採集對 *Phytophthora capsici* 及其所引起的病害有抑制作用的土壤，以高壓蒸氣殺菌或在 500C 高溫處理十六小時後，這個土壤的抑菌作用仍舊存在。抑菌因子存在於土壤中的粘粒，坍粒及砂粒中，但不存在於水的抽出液中，由此可知 *P. capsici* 是被抑菌土中的無機顆粒所抑制。

ABSTRACT

There was an inverse relationship between percentage of germination of *Pythium splendens* sporangia and soil pH among the soil samples tested. A combination of high Ca content and high total microbial population was found to be responsible for inhibition of *P. splendens* in a suppressive soil.

There was a direct relationship between relative hyphal growth of *Rhizoctonia solani* and soil pH among the soil samples tested. Inhibition of *R. solani* in a suppressive soil was shown to be the result of microbial activity and high Al ion concentration.

A forest soil suppressive to *Phytophthora capsici* and the disease caused by the pathogen remained suppressive after autoclaving or ignition at 500°C for 16 hr. The inhibitory factors existed in the fractions of clay, silt and sand but not in the aqueous extract of suppressive soil, suggesting that inorganic soil particles are responsible for inhibition of *P. capsici* in this suppressive soil.

INTRODUCTION

Lower incidence of soil-borne disease in a given field compared with that of a nearby area has been observed frequently by growers with inquiring minds. However, the number of reports concerning such natural ways of controlling soilborne diseases so far has been very limited[1,2,9]. The facts that the fungistasis potential varies greatly among different soils[3] and that both volatile and non-volatile inhibitory substances exist in certain soils[4,11,12,13] strongly suggest that disease- and pathogen-suppressive soils may be relatively common in nature and may also exist in small islands in a conducive field. Both possibilities have been shown to be correct when a large number of soils were tested with a simple and rapid method developed to detect the inhibition of pathogens in a small amount of soil.[10] In 109 soil samples collected from the islands of Hawaii, Maui, Molokai and Kauai, about 40% were suppressive to *Pythium splendens* and 10% to the disease caused by this fungus[6]. For *Rhizoctonia solani*, about 14% of the 111 soil samples tested were suppressive to the pathogen and 5% to the disease caused by this fungus[5]. Among the five soil samples collected from a three-acre area of uniform soil texture at Lalamilo Experimental Farm, one was suppressive and four were conducive to *P. splendens*. In another experiment three out of 12 soil samples collected from a one-acre area of pasture at South Kohala were suppressive to *R. solani* and the other nine were conducive, although all were suppressive to *P. splendens*[10]. These results confirm the suggestion that suppressive soils may exist in small islands in a conducive field.

PYTHIUM SPLENDENS-SUPPRESSIVE SOIL

Sporangia of *P. splendens* suspended in cucumber root extract to overcome the general fungistasis were placed on the smooth surface of a soil block on a glass slide and stained with rose bengal after incubation at 24°C for 6 hr. Germination was counted directly on the soil surface using a vertical illumination microscope[10]. There was an inverse relationship between percentage of sporangial germination of *P. splendens* and soil pH when 31 soil samples collected on the island of Hawaii were analyzed. A similar relationship was obtained when 81 soil samples from the islands of Hawaii, Maui, Molokai and Kauai were analyzed. These results suggest that near neutral to alkaline soils in Hawaii tend to be more suppressive to *P. splendens* than acid soils[6].

A pasture soil in South Kohala which was highly suppressive to *P. splendens* and damping-off of cucumbr caused by the pathogen was selected for studying the mechanism of pathogen suppression. The suppressive soil remained suppressive when its pH was adjusted from the original 6.8 to 5.4 of the conducive soil, indicating that soil pH is not a contributing factor. Several lines of evidence suggest that non-specific microorganisms are involved in suppression of *P. splendens* in South Kohala soil[7]:

1. Inhibition of *P. splendens* by suppressive soil decreased with increasing depth of soil which was associated with decreasing populations of

bacteria, actinomycetes and fungi.

2. Sterilization by autoclaving or gamma-irradiation, or reduction of microbial population by steam treatment, completely eliminated the inhibitory effect of suppressive soil. Inhibition was restored to sterilzed suppressive soil by reinfestation with a suspension of either suppressive or conducive soil, or isolated bacteria, actinomycetes or fungi.
3. Inhibition was partially nullified by amendment of suppressive soil with rose bengal, streptomycin (both inhibitory to bacteria and actinomycetes) or benomyl (inhibitory to fungi).

Although microorganisms are associated with suppressiveness of South Kohala soil, the microbiological factor alone is insufficient to produce the inhibitory effect because of the following observations[3]:

1. Infestation of autoclaved conducive soil with suppressive soil suspension did not render its inhibitory.
2. Colonized gamma-irradiated conducive soil remaind conducive even though its total microbial population was higher than that in natural suppressive soil.
3. Adjustment of total microbial population in conducive soil to the same level as that in suppressive soil did not render it inhibitory.

These results suggest that a combination of biotic and abiotic factor are responsible for the suppressive effect of the South Kohala soil.

Since *P. splendens* suppression in Hawaii usually occurs in high pH soils some of which are known to contain high Ca content, pathogen suppression was compared with Ca content and total microbial population in suppressive and conducive soils. It was found that inhibition of *P. splendens* in suppressive soils was associated with high Ca content and high total microbial population. Amendment of conducive soil with $CaCO_3$, or increasing the microbial population in conducive soil by adding pure cultures of microorganisms or by amending the soil with alfalfa meal only slightly affect sporangial germination of *P. splendens*. However, when conducive soil was amended with $CaCO_3$ and microorganisms or $CaCO_3$ and alfalfa meal, germination of *P. splendens* decreased from 89% to 46% and 18%, respectively[8]. Stored soil and subsoil from South Kohala are high in Ca content and low in microbial populaton and are not inhibitory to *P. splendens*. Increasing the microbial population by adding water to stored soil or amending subsoil with alfalfa meal decreased the germination of *P. splendens* from 93% to 10% and 86% and 24%, respectively. The inhibition characteristics of artificially induced suppressive soil (conducive soil plus $CaCO_3$ and alfalfa meal) were similar to those of natural suppressive soil. It is therefore suggested that a combination of high Ca content and high total microbial population is responsible for inhibition of *P. splendens* in South Kohala soil[8].

RHIZOCTONIA SOLANI-SUPPRESSIVE SOIL

A polycarbonate membrane disc containing mycelia of *R. solani* was placed at one end of a soil block in a large plastic petri dish. Linear growth of

R. solani hyphae on the soil surface was recorded under a stereomicroscope every two days for four days[10]. Relative hyphal growth of *R. solani* on each soil was calculated as a percentage of hyphal growth on conducive Hilo soil. There was a direct relationship between relative hyphal growth of *R. solani* and the pH value of soil when 60 soil samples collected from the island of Hawaii were analyzed. A similar relationship was obtained when 111 soil samples collected from the islands of Hawaii, Maui, Molokai and Kauai were analyzed. These results suggest that acid soils in Hawaii tend to be more suppressive to *R. solani* than near neutral to alkaline soils[5]. The Onomea forest soil from the island of Hawaii, which was suppressive to *R. solani* and damping-off of mustard cabbage caused by the pathogen, was selected for studying the mechanism of inhibition.

When conducive soil was mixed with 25,50 or 75% of suppressive soil, relative growth of *R. solani* on conducive soil decreased with increasing amount of suppressive soil. However, growth inhibition in conducive soil did not increase during the incubation period, indicating that the suppression factor did not increase in the conducive soil; therefore, it is not transferable.

Inhibition of *R. solani* in suppressive soil is associated with microbial activity because autoclaving the soil eliminated the inhibitory effect and the inhibition was restored to autoclaved soil by reinfestation with soil microorganisms. Suspensions of both suppressive and conducive soils were capable of restoring inhibition to autoclaved suppressive soil but not autoclaved conducive soil, indicating that inhibition of *R. solani* in Onomea soil is the result of a combination of nonspecific microorganisms and an abiotic factor[5].

When the pH of suppressive soil was adjusted from the original 4.2 to 7.0, the relative hyphal growth of *R. solani* increased from 16% to 105%. The suppressiveness was not restored to the soil when its pH was readjusted to 4.2. Damping-off of mustard cabbage caused by *R. solani* increased from 26% to 100% when the pH of suppressive soil was adjusted from 4.2 to 7.0.

Extracts of suppressive soil but not conducive soil were inhibitory to hyphal growth of *R. solani*. The inhibitory effect also disappeared when the pH of the soil extract was adjusted from the original 4.2 to 7.0. Since germination of *Neurospora tetrasperma* was also inhibited in suppressive soil extract but not conducive soil extract and it required only 6 hr for each test rather than four days using the hyphal growth of *R. solani*, it was used as a bioassay in subsequent tests. Ascospores of *N. tetrasperma* germinated 33 to 41% in suppressive soil extracts and 93% in conducive soil extract. The Al ion concentration in the suppressive soil extracts ranged from 3 to 5.6 ppm, while that in the conducive soil extract was 1.6 ppm. Germination of *N. tetrasperma* ascospores in water containing 0,5 and 10 ppm $AlCl_3$ was 92,35 and 0%, respectively. These results suggest that Al ions may be an abiotic factor contributing to the inhibition of *R. solani* in Onomea soil[5].

PHYTOPHTHORA CAPSICI-SUPPRESSIVE SOIL

Sporangia of *Phytophthora* usually germinate indirectly by releasing zoospores in the absence of nutrients and directly by producing germ tubes in the presence of nutrients. Therefore, *P. capsici* sporangia were preincubated in 50% V-8 juice for 1 hr before being added to smooth surface of soil block to ensure uniform direct germination. A forest soil collected from the island of Hawaii was found to be strongly suppressive to sporangial germination of *P. capsici* and damping-off of tomato seedlings caused by the pathogen. Increasing the nutrient concentration in the sporangial suspension before addition to the soil did not increase sporangial germination, indicating that nutrients were not effective in overcoming the inhibitory effect of suppressive soil. When the pH of suppressive soil was adjusted from the original 4.2 to 7.0, sporangial germination of *P. capsici* increased from 3% to 35%. Adjusting the pH of coducive soil from the original 7.0 to 4.2 also decreased the germination from 92% to 30%. These results suggest that, although low pH is inhibitory to *P. capsici*, it alone cannot account for the strong inhibitory effect of the Hawaii soil against this pathogen [14].

Subsoils collected at depths of 30, 45, 60, and 75 cm from the suppressive area were also strongly inhibitory to sporangial germination of *P. capsici*. The suppressive soil was still inhibitory to *P. capsici* after autoclaving, indicating that living organisms were not responsible for the suppression. The inhibitory effect of the suppressive soil was not decreased after ignition at 500°C for 16 hr or treatment with H_2O_2 to remove organic matter. These results suggest that the inhibitory factor in the suppressive soil is inorganic and is heat stable.

To determine if the inhibitory factor is water soluble, 25 g of suppressive soil was shaken with 25 ml of water for 2 days[12]. The extract was not inhibitory to sporangial germination of *P. capsici*. When *P. capsici* sporangia were placed on a polycarbonate membrane (0.2 μm) on the soil, germination increased from 1% without membrane to 92% with membrane, suggesting that the inhibitory factor in the suppressive soil is not diffusible. The fractions of clay, silt and sand of the suppressive soil were all inhibitory to *P. capsici*. Results suggest that inorganic soil particles are responsible for the inhibition of sporangial germination of *P. capsici* in the suppressive soil[11].

DISCUSSION

Through extensive survey of pathogen-suppressive soils in nature in Hawaii, and intensive study of inhibition mechanisms of some of these suppressive soils, several important features concerning such natural ways of controlling soilborne diseases have been revealed:

1. Soils suppressive to different pathogens appear to exist commonly in nature.
2. Suppressive soil may cover the whole field. It may also be localized to form small patches in a conducive field.
3. Abiotic components of soil play an important role in pathogen inhibition in different suppressive soils.
4. Different pathogens may be inhibited by different mechanisms in the sup-

Table 1. Nature of soils suppressive to pathogens in Hawaii

	Soil suppressive to		
	Pythium splendens	*Rhizoctonia solani*	*Rhytophthora capsici*
1. Soil			
Classification	Ustollic Eutrandepts	Typic Hydrandepts	Typic Hydrandepts
Type	Very fine sandy loam	Silty clay loam	Silty clay loam
pH	6.8	4.2	4.2
2. Treatment			
Autoclaving	C[1)]	C	S
Nutrient amendment	C	N.T.[2)]	S
pH adjustment	S[1)]	C	M.S.[1)]
3. Mechanism			
Microorganisms	Nonspecific	Nonspecific	Not involved
Abiotic factor	Ca	Al^{3+}	Inorganic particles

1) S: Suppressive; M. S.: Moderately suppressive; C: Conducive.
2) N. T.: No test.

pressive soil (Table 1).

The general application of these findings regarding suppressive soils remains to be investigated in other parts of the world.

A suppressive soil like a sick plant is a general term. Description of symptoms of each sick plant is important to the distinction of one sick plant from the others, be it a wilt, blight, canker or mosaic, likewise description of inhibition characteristics of each suppressive soil is essential for its identification.

Studies of the mechanism(s) of suppression of a pathogen in a suppressive soil ultimately require experimentation whereby a conducive soil is made suppressive. Such experimentation may involve transfer of a specific organism or group of organisms, alteration of population of indigenous microorganisms, or adjustment of physical and chemical properties of soil or a certain combination of these. It is essential in such studies however that criteria be used to make certain that any suppressive soil created artificially operates by the same mechanism(s) operative in the same natural suppressive soil. There are many ways a plant may be diseased, similarly there are probably numerous ways a pathogen can be suppressed in the soil. Just as completion of Koch's postulates for establishing the pathogenicity requires the reproduction of the exact symptoms of a disease, so completion of the equivalent of a "Koch's postulates" for establishing the suppression mechanism(s) will require that the suppressiveness created is exactly the same as that operating naturally. This major step will depend on a careful comparison of the inhibition characteristics of natural and created suppressive soils.

Distinct inhibition characteristics of pathogen-suppressive soil may include:

1. Inhibition spectrum. Effect of a suppressive soil on different microorganisms could range from inhibitory to stimulatory[7].
2. Effect of pH adjustment. Certain suppressive soils partially or completely lost the inhibitory effect after adjustment of soil pH[2,5,14], while the inhibitory effect of others was not affected by pH change[7].
3. Effect of high-temperature treatment. Although most suppressive soils lost the suppressiveness after heat treatment[2,5,7], the inhibitory effect of some suppressive soils was not decreased by the same treatment[15,14].
4. Response to selective inhibitors. Different types of suppressive soils may respond differently to the same selective inhibitors[2,7]. Response of a suppressive soil to certain selective inhibitors also indicates the association of certain group(s) of microorganisms with the inhibitory effect of the suppressive soil.
5. Response to nutrient amendment. Nutrients may or may not counteract the inhibitory effect of a suppressive soil depending on the nature of the suppression[2,14].
6. Mode of inhibition. The inhibitory effect of a suppressive soil on the pathogen may be either fungistatic or fungitoxic[2,14].
7. Effect of separation by polycarbonate membrane. Whether the pathogen will be inhibited on suppressive soil when it is separated from the soil particles and microorganisms by a polycarbonate membrane (0.2 μm) can also be used as a distinct characteristic of a suppressive soil.

Similar approaches can be used to characterize a disease-suppressive soil.

These or other criteria have not been followed in other studies of the nature of inhibition in suppressive soils[1,2]. For example, Liu and Baker[15] found that soil repeatedly planted with radish became suppressive to *Rhizoctonia solani*. This was associated with increase in population of *Trichoderma* spp. which when introduced into conducive soil induced suppressiveness. However, other factors have not been ruled out and the characteristics of the *Trichoderma*-induced suppression have not been compared with that of the repeated planting. Therefore, one cannot be sure that the suppression induced by added *Trichoderma* is the induced by repeated planting.

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